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TITEL: AUTOMATED MECHANICAL STRESS ASSAY FOR SCREENING CLEANING INGREDIENTS

FIELD OF THE INVENTION

This invention relates to an automated assay for testing real application performance of cleaning ingredients by testing them on mechanically stressed surfaces. The invention also relates to surfaces and other devices adapted for application in the automated assay and to cleaning ingredient identified by the assay and subsequently produced.

BACKGROUND OF THE INVENTION

It has been the object for researchers in the detergent industry to continuously search for new ingredients for use in cleaning compositions, which improves the cleaning effects of these compositions. In the field of surface cleaning, such as textiles of fabrics most people believe, that the flow of a liquid cleaning solution also called the washing liquor against and/or through a surface is sufficient for achieving a good cleaning process and accordingly that such conditions are suitable when the cleaning effect of new cleaning ingredients and/or compositions are evaluated to identify improvements.

Various artificial methods has over time been developed to provide tools for simulating a cleaning process and for testing cleaning effects. However using artificial methods often results in the finding of promising cleaning ingredients or compositions, which despite the predictions from the artificial methods does not provide the expected improvement when applied to real cleaning processes. Accordingly, in order to test and make reliable predictions on the cleaning effect of new ingredients or compositions, the skilled person has had adopt full scale cleaning processes and real surfaces, such as cleaning fabrics in a washing machine to provide useful results.

WO 99/34011 describes a method for assaying wash performance of new enzymes and/or detergent formulations.

The published Danish patent application PA 1997 00507 describes an assay for analyzing cellulolytic detergent enzymes.

SUMMARY OF THE INVENTION

Our research has shown that, besides contact between washing liquor and a fabric, the mechanical stress, which in real life applications is conferred to the fabric, is also of major importance. However, full scale testing methods are difficult to automate and has a very limited sampling capacity rendering these method expensive and laborious. With the present invention we are able of remedying these limitations by providing

A method for testing cleaning effect of a compound or compositions thereof said method comprising:

(a) Preparing a liquid sample of less than 10 ml comprising the test compound,
(b) applying liquid sample to a stained surface,
(c) applying mechanical stress to the stained surface by contacting it with a body present in the liquid sample,
(d) evaluating the cleaning effect of applying solution and mechanical stress on the stained surface.

As this method is particularly suitable for automated testing of small volume samples, the invention also relates to a device developed for testing cleaning effect of a composition, said device comprising:

(a) at least one container having a volume of less than 10 ml, preferably less than 2 ml, most preferably less than 0.2 ml,

(b) at least one body capable of moving inside the container,

(c) at least one stained surface and

(d) means for providing movement of the body relatively to the stained surface.

The invention in accordance with these aspects provides means easily automated for testing of cleaning compositions, means which, through the use of small test samples less than 10 ml, preferably less than 2 ml, most preferably less than 0.2 ml , offers a large sampling capacity and through the element of mechanical stress offers simulation of the cleaning process comparable to real life or full scale cleaning processes.

BRIEF DESCRIPTION OF THE TABLES AND DRAWING

Figure 1 is a drawing showing the principle of using a permanent magnet for providing mechanical force to a stained fabric.

Figure 2, shows the results of testing effect of a xyloglucanase enzyme on a stained fabric in a test method using a permanent magnet for providing mechanical force to a stained fabric.

Figure 3 is drawings showing cross sections of different types of assemblies suitable for use in testing effects of cleaning ingredients.

Figure 4 shows a cross section an assemble comprising a micro plate fitted with a lid and a stained fabric, the assembly being suitable for use in testing effects of cleaning ingredients.

Figure 5 shows an assembly such as given in figure 4, mounted on a device for generating mechanical force.

Figure 6 shows the results of testing different protease enzymes in a commercial 96 well test plate with a single coherent stained fabric covering all well openings of the commercial plate, the test plate and stained fabric mounted on the device of figure 5 without addition of a movable body to the wells.

Figure 7 shows the results of testing different protease enzymes in the a 96 well custom made test plate assembly according to figure 4 with a single coherent stained fabric covering all well openings of the plate, the test plate and stained fabric mounted on the device of figure 5 without addition of a movable body to the wells.

DETAILED DESCRIPTION OF THE INVENTION

The method

One object of the invention is to provide a method for testing the cleaning effect of compounds on a stained surface using only small sample volumes less than 10 ml while, but which simulates and is comparable to real cleaning processes performed e.g. in a washing machine. One challenge in developing such a method is the implementation of the mechanical stress factor in a small sample volume system. It is to be understood that the system is a collection of functional members or tools used to carry out the method of the invention such as containers, stained surfaces, bodies or samples, and/or means for sealing, temperature control, pressure control etc. as described, *infra*.

The method we have developed, simulating full scale cleaning of surfaces, is easily automated and it is possible and even feasible to employ conventional testing vessels e.g. arrays of small containers, such as wells of a micro plate. The method and devices developed for performing the method allows to simulate cleaning processes on surfaces like fabrics at a micro

scale even using extremely small sample volumes such as below 50 µl. The evaluation of cleaning effect of compounds such as new enzymes, can be done using this method at a 50.000 to 500.000 times smaller quantities than it is required for a full scale cleaning process. Basically the method provides a predictive system with the capability to be incorporated in high throughput facilities as it is established in the pharmaceutical industry. This new tool can therefore accelerate dramatically the development effort of new and improved cleaning compounds and/or compositions by providing the capability to test ingredients at high numbers and of low quantities.

The test compound

The purpose of the present invention is to provide means of identifying compounds or compositions thereof providing improved cleaning of a stained surface.

The compound or compositions thereof in the context of the invention is preferably selected from compounds known to be incorporated in detergent compositions such as enzymes and/or enzyme stabilizers, inhibitors, enhancers, co-factors, builders, builder systems, bleach systems, bleach activators, metal-containing bleach catalyst, optical brighteners, , nonionic -, anionic -, cationic -, zwitterionic and amphoteric surfactants, fabric softening agents, softening clays, clay flocculants, dye-transfer inhibiting agents, polymeric soil release agents, clay soil removal agents, anti-soil redeposition agents, polymeric dispersing systems, chelating agents, alkoxylated polycarboxylates, perfumes, perfume systems, carrier systems, dyes and pigments, fabric care agents, color care agents and like.

Typical enzymes used in fabric care and cleaning products are proteases, lipases, amylases, cellulose hydrolyzing enzymes such as cellulases, carbohydrases, transferases, oxidoreductases and like, preferably the enzymes are alkaline enzymes

characterized by having their catalytic optimums between pH 7-12, more preferably high alkaline having their catalytic optimums between pH 9-12.

The liquid sample

In the present invention a liquid sample is prepared comprising the compound and/or the composition to be tested. The sample is preferably an aqueous solution or dispersion of the test compound or composition, but it may also be non-aqueous of nature, which may be relevant when testing compounds suitable for use in non-aqueous cleaning liquids.

In a preferred embodiment the liquid sample is prepared by first preparing a base solution and/or dispersion and transferring predefined amounts hereof to a test vessel or container. The compound or composition may then be added to the vessels in minute amounts, preferably in solution. In accordance with the invention, the liquid sample thus prepared have a volume of less than 10 ml, preferably less than 2 ml, most preferably less than 0.2 ml , but the method works with considerably less volume, which is feasible to increase the sample capacity. Accordingly the sample volume is comparable to the well volume of commercially available micro plates. A suitable volume is less than the well volume of a 24 well micro plate, preferably less than the volume of a well on a 96, 384 or 1536 well plate. Based on the type of micro plate, the volume can be chosen between 5 - 95% of the volume of the well, which is 3.7 ml, 320 μl, 160 μl, and 14 μl, respectively.

The surface

Surface materials relevant to be employed in the present invention are materials used in real life, which are subjected to cleaning processes. Such materials may be inorganic such as metal, ceramic, glass, enamel concrete, rock, marble, gypsum or composite combinations thereof. The material may also be mainly

organic by nature such as plastic, rubber, wood, paper, leather, fur, paint or fabric. A preferred surface is a fabric. The fabric may be any fabric made from natural plant fibers, animal based fibres or synthetic fibres or combinations thereof. The fabric may be woven or non-woven or soft or stiff. Most preferred fabrics are cellulose containing fabrics such textiles (woven) and tissues (non woven) and animal based fabrics such as wool.

A stained surface may be achieved by employing any conventional staining techniques associating a stain comprising a traceable compound or composition to the surface and/or associating the traceable compound or composition itself to the surface.

Suitable traceable compounds includes dyes such as light absorbing or fluorescent dye, radioactive compounds, reactive compounds, such a catalysts and/or activators capable of performing measurable interaction with substrates. Such compound may be associated directly or indirectly to the surface by covalent bonding, ionic bonding and/or hydrogen bonding.

The surface may also be stained with a particulate composition such carbon particles, e.g. carbon black or iron oxides.

In a preferred embodiment the surface is stained by a soiling composition comprising the traceable compound or composition. Such soiling composition is preferably a naturally occurring soiling such as grass, mud, clay, coffee, tea, blood, egg, lard, moulds (damp stained) or the composition may made of processed naturally occurring soiling, such as butter, processed meat, dyed lard, oil, make up, spice blends, processed tomatoes (ketchup or puree), chocolate, ice cream, cacao, baby food and the like. The soiling may also preferably be a man made composition comprising compounds

selected from refined protein compositions, refined polysaccharide compositions, refined fatty acid compositions, refined triglyceride compositions or other refined biological or non-biological compounds.

Most fabrics are stained by applying the staining material as it is or as an aqueous solution onto the fabric surface by soaking, brushing and/or spraying. The stained fabrics will be dried before testing. There is also a group of colourless materials, which bind soil and particles onto surfaces. Carbohydrates like Guaran Gum, Locust Bean Gum and starch and the like are belonging to this group of materials, which are attracting soil materials. One example of preparing stain with such materials is as follows:

1) Dissolving the soil binding material Guar Gum in a aqueous solution at a level of 0.1 to 1 %.
2) Applying the solution onto the fabric surface by soaking, brushing, or spraying onto the surface.
3) Removal of the access gum material by washing the coated fabrics in a commercial washing machine (Miele, short cycle, 40°C) without detergent.
4) Staining of the coated fabrics in a commercial washing machine (Miele, short cycle, 40°C) by adding 0.1 to 1% of clay, carbon black, iron oxide and/or other pigments/particular soiling materials into the wash machine without detergent.

Fabric surfaces having a range of different stainings are commercially available under the trade name EMPA® swatches marketed by EMPA St. Gallen, Lerchfeldstrasse 5, CH-9014 St. Gallen, Switzerland.

The body

For applying mechanical stress to the surface the present invention requires a body present in the liquid sample comprising the test compound. It is necessary that the body has

a size and shape which allows the body to go into the container holding the liquid sample, so that the body is present in the liquid sample. Preferably the body has a size and a shape, which allows the body to move in multiple directions within the container holding the sample and the body. The size and shape of the body should also preferably allow the body to be submersed in the sample.

The body is made of a material, which is preferably insoluble in the solution. The body is preferably rigid, but it may also be flexible and it may have a smooth or a rough surface. It is important that the body material is predominately inert, i.e. that the body does not chemically interact with the sample or vice versa. Accordingly the body material is made from durable materials. Such materials may be inorganic such as metal, ceramic, glass, enamel concrete, rock, marble, gypsum or composite combinations thereof or organic such as plastic, rubber or combinations thereof. Preferably the material is sufficiently ductile to prevent breakage of the body upon collision with the stained surface or container walls. The body may also be any suitable material coated with said inert and ductile material.

In a preferred embodiment the body comprises a metal, more preferably a ferromagnetic metal such as iron or alloys thereof or a composite comprising such metals or alloys. To inhibit corrosion of metals, the body is preferably coated with a layer of inert material such as organic polymers, separating the metal(s) from any outer corrosive media.

In this case the body may be any suitable nut, bolt, screw, bed or ball, preferred bodies have a surface comprising at least one edge or corner like a rectangular body.

Any shape like spherical, round-flat, disk shape, cube shape, elongated cube shape, cylindrical round shape, cylindrical rectangular shape, star shape, hexagonal shape,

and like is suitable which fulfils the requirements for size and shape to fit into the test container.

The choice of body material is important for the choice of method for transferring kinetic energy (movement) to the body. The size, shape, mass and surface texture is important for the type and amount of mechanical stress the body is capable of conferring to the stained surface.

Depending on the type of compound to be tested excellent results have been obtained with 3-5 rectangular stainless steel bodies (3 x 1.6 x 1.9 mm) per container in a 96 well plate or stainless steel balls or beads having a diameter of 3 mm or less.

Application of liquid sample and mechanical stress to the stained surface.

In the method of the invention the stained surface must be placed where the body and the sample solution has access to the stained surface. Having the sample and the body in a container the stained surface may be placed in any position from the bottom to the top of the container. Preferably the stained surface is placed at the top or the bottom of the container and more preferably the stained surface functions as a cover on an opening in the container.

When the stained surface is placed below sample level in the container (submersed), the application of the liquid sample to the stained surface is continuous, while when the stained surface is placed above sample level in the container application of the liquid sample to the surface depends on movement of liquid sample relative to the stained surface to allow liquid to contact the surface. This may be achieved by movement of the liquid, movement of the stained surface a combination thereof.

The mechanical stress may be conferred to the stained surface by providing movement of the body relative to the

stained surface, which allows the body to collide with the stained surface. This may be achieved by movement of the body, movement of the stained surface a combination thereof.

In the preferred embodiment of the stained surface acting as a lid on top of the container, the application of sample liquid and mechanical stress to the stained surface may advantageously be combined. In this case the mechanical stress may be provided to the to the stained surface by moving the body and allowing it to collide with the surface. However, as the body moves from having contact with the liquid sample, e.g. by being submersed therein, some sample liquid will adhere to the body and when the body collide with the stained surface some of liquid sample adhering to the body will be deposited or applied on the surface. Further, repeating movement and collision between surface and body will provide exchange of previously deposited sample and fresh sample, so that changes in the sample liquid deposited on the stained surface, e.g. as a result of interaction between stain and sample liquid, will be equilibrating with un-deposited sample liquid. Accordingly, by employing this system application of both liquid sample and mechanical stress may be achieved by moving the body. This system further solves problems related to having the stained surface present inside the container, e.g. submersed in the liquid sample. Firstly, such submersed surfaces has to be cut into very small pieces to fit into the containers encompassed by the invention, which may be a tedious procedure, secondly, a submersed surface may interfere with movement of the body, as stained surface and body may get entangled with each other inhibiting or changing the movement pattern, third, the mechanical stress conferred to the stained surface by collision with the body may occur in an irregular and non-reproducible manner, introducing increased levels of noise into the evaluation of the cleaning effect, fourth, if evaluation of the

cleaning effect is includes analyzing the stained surface this process is difficult to automate because each stained surface is to handled individually. The second, third and fourth problem may, however, be somewhat remedied by attaching the stained surface to the bottom of the container, which may be feasible if the method for evaluating the cleaning effect of the liquid samples allows such a setup, e.g. if measurement can be made on the liquid sample or on the stained surface through the bottom wall of the container.

Movement of liquid sample, the body and/or the stained surface may be provided for by employing a force, preferably an oscillating force. The term oscillating as used in this context means that the force is following a directional and amplitudical repetition scheme, preferably a periodical pattern, in amplitude and direction. As an example an applied force may repeatedly act first upwards and then downwards on the body (or any other element of the invention) and/or it may act first towards one side and the towards the opposite side and/or from front to back. The force might be applied in a regular, periodic oscillating manner or completely in random, non-periodic irregular manner. An oscillating force also encompasses combinations of these directional forces, i.e. in altering force patterns such as applying upward and downwards force for defined period of time followed by applying side wards forces for period of time followed again by up and downwards forces and so on. By applying an oscillating force the collisions between body and stained surface are repeated many times conferring significant mechanical stress to the stained surface.

Various types of forces may be employed to achieve movement of the liquid sample, the body and/or the stained surface.

The oscillating force may be mechanical of nature e.g. by applying external movement to all or selected parts of the system, which subsequently confers movement to the liquid sample, the body and/or the stained surface. This may be achieved by subjecting the system or parts of the system to shaking and/or vibration, e.g. by vibrating the container holding the liquid sample, the body and the stained surface. Mechanical forces includes subjecting the system or parts of the system to motion waves such as sound waves, preferably of higher frequency such as ultra sonic waves.

Other suitable forces are forces capable of acting on selected system elements having special properties. Such forces include magnetic, electromagnetic, electrostatic and/or electrical forces. Magnetic or electromagnetic forces for example act only on magnetizable materials such as ferromagnetic materials. Accordingly it is possible to employ forces, which acts only on selected elements of the system. One advantage of applying these types of forces is that these forces act on susceptible elements though force fields, which may act on susceptible materials over distance penetrating non-susceptible materials. Accordingly it is possible the apply force to a system element, such as the susceptible body, e.g. through a non-susceptible container wall. As an example a magnetic force field will act on an ferromagnetic containing body through the wall of a plastic, glass or non-ferromagnetic metal container.

In a preferred embodiment the force includes at least one force field selected from magnetic field, electromagnetic field, electrostatic field and electric field.

In a further preferred embodiment is preferred to employ a force acting on and moving the body only whereby, as described *supra*, both application of liquid sample and mechanical stress to the stained surface may be achieved independent of the position of the surface. More preferably the force includes a

magnetic or electromagnetic field and the body comprises a magnetically susceptible material, such as a ferromagnetic material. It is to be understood that in this embodiment, the force may also act on components of the liquid sample on a molecular level, such as ions or it may act on fixed members of the system elements, but it does not directly induce movement of the liquid sample, the stained surface, the container or other elements (such as tables, container holder etc.) used to carry out the method of the invention.

The present invention also encompasses employment of combinations of different force types such as combination of mechanical force and magnetic force fields.

In a most preferred embodiment the force is applied to a magnetizable body by moving a magnet relatively to the container containing the body, thus altering the magnetic field affecting the body or the force is a mechanical vibration force applied to an assembly comprising body, container and stained fabric.

A suitable system consists of a micro plate and a source of field force on top of the plate. A modification is an additional force field at the bottom of micro plate.

Evaluation of cleaning effect

Evaluation of the cleaning effect of a sample liquid may be achieved by analyzing the stained surface, the liquid sample or both after subjecting the stained surface to the liquid sample and the mechanical stress. A suitable choice of method will depend on the type of stain, which is used.

It may be feasible to measure changes of either surface or the liquid sample or both. Various methods are known to the art and includes optical methods, optical methods combined with coupled reaction between staining compounds and indicators, enzyme assays, physical measurements of altered surface properties chromatographic or fluorimetric methods, spectroscopic methods, radioactive labeling methods,

immunochemical methods such as ELISA and more. If the stain is a more or less complex soiling composition it is usually more feasible to analyse the stained surface e.g. by measuring the stained surface reflectance of light with suitable reflectometers or colorimeters.

The testing device

As described, supra, this invention also relates to a device suitable for testing cleaning effect of a composition, said device comprising:

(a) at least one container having a volume of less than 10 ml, preferably less than 2 ml, most preferably less than 0.2 ml,
(b) at least one body capable of moving inside the container,
(c) at least one stained surface, preferably a stained fabric and
(d) means for providing movement of the body relatively to the stained surface.

The advantageous features of speed and capacity in testing compounds provided by the present invention relies to a major extent on the assembly of equipment making up the testing device. Accordingly, an important aspect of the invention is the practical applicability of the stained surface. In order to achieve the optimal capacity and speed in the present assay it is, as described *supra*, preferred to use a stained surface which functions as a lid on the container in which the test sample is placed. Within this embodiment it is further preferred that the stained surface, as a coherent entity, has a dimension, which makes it capable of acting as a lid on two or more containers simultaneously. More preferably the stained surface have a dimension, which makes it capable of acting as a lid on an array or arrays of containers simultaneously enables. The embodiment of using a stained surface having a dimension, which makes it capable of acting

as a lid on numerous test containers is very important indeed not only considering test capacity but also considering the accuracy of the testing. For many stained surfaces, such as fabrics, there may be variations between different entities of the same type of surface. For example variations in the texture and firmness between different parts of a cloth, from which stained fabric sub-pieces are made, may cause variations between fabric sub-pieces in stainability and ability to interact with test compound(s). However, by choosing a test assembly which requires that the stained fabric has a certain dimension, which, as a coherent entity, is capable acting as a lid for numerous test containers, it is secured that the stained fabric used to test compounds in each individual container has a lowered variation in texture, firmness and staining, because it, taken as a coherent entity must originate from the same part or area of e.g. the cloth from which it is taken. The term "coherent" in this context is to be understood in the broadest sense, but preferably that the fabric is in one coherent woven or non woven piece without seams or stitches or other fastening means, which may make the fabric surface less uniform.

Accordingly, the present invention also relates to an assembly suitable for use in testing effect of cleaning ingredients, e.g. in the device as described above, which through a special combination of containers and stained fabric provide for high speed and capacity as well as good accuracy in testing cleaning ingredients, such as enzymes. Accordingly, the present invention provides an assembly comprising at least one container and a stained coherent fabric, wherein the container comprises at least one opening covered and/or lidded with the stained coherent fabric. The invention also encompasses an assembly comprising a container and a stained coherent fabric, wherein the container comprises two

oppositely positioned openings and at least one, preferably both of said openings are covered and/or lidded with a stained coherent fabric. In one preferred embodiment the assembly further comprises least one body in each container for providing mechanical stress to the stained fabric, as described *supra*, preferably within the container. In another preferred embodiment the assembly comprises an array of containers, wherein each container comprises one or more openings and optionally one or more bodies and wherein the stained coherent fabric covers and/or lids at least one of the openings in each container. In a further preferred embodiment the array of containers is a micro plate comprising 24, 96, 384 or 1536 containers or wells and the stained coherent fabric have a preferred dimension within 0.2-10 cm by 0.2-15 cm, preferably a dimension enabling the stained fabric to cover all the wells in the micro plate.

The term "cover/lid" in this context is to be understood in the sense that the stained fabric covers the opening of a container by being in contact with the edges of the opening.

Preferably the assembly comprises an additional support lid or cover located in the assembly so that the fabric is placed between the support lid and the container opening. The support lid is preferably made from a material, which is impermeable to the liquid sample in the container and has a dimension which makes the support lid rigid enough to support the fabric and prevent the fabric from moving away from the opening when subjected to mechanical force. The assembly also preferably comprise means for fastening the support lid to the container so that the fabric may act as a sealing gasket. One important feature in this context is that the contact zone between the fabric and the edge of the opening is small so that the pressure holding the fabric in contact with the container opening, when the support lid is fastened to the

container, is sufficient to seal the container, using the fabric as a gasket. This is important to prevent or inhibit that liquid sample from one container contaminates, through capillary movement, an adjacent container. To improve contact pressure between fabric and container opening by minimizing the contact zone, the edge of container openings and interconnecting surface between containers and the side of the support lid facing the fabric are designed so that they form a line toward each other instead of a surface. This can be achieved by designing the support lid and containers so that the interconnecting surface between containers in an array and the surface of the support lid facing the fabric are essentially unparallel, i.e. greater or less than about 180°. Examples of this design is given e.g. in figure 3.

Also other elements may be added to the assembly, such as devices for providing mechanical force to the body, the sample, the fabric or a combination, means for controlling temperature, devices for measuring properties of the stained fabric or the test sample. For convenience the assembly comprising container, stained fabric and optionally a support lid is from hereon referred to as the "primary assembly" and assemblies comprising additional elements are referred to as "secondary assemblies". Accordingly the present invention encompasses any test system comprising primary or secondary assemblies.

In one embodiment of the invention a secondary assembly comprises a device for providing mechanical force, the device preferably comprises a piston in a cylinder construction for generating vibration or shaking force, which is transferable to the stained fabric. Preferably the piston device is connected, via a rigid plate, suitable for mounting and/or fastening the primary assembly, to the primary assembly. The piston based device is preferably driven by a pneumatic or hydraulic system, using compression and/or expansion of air or

liquid to move the piston relative to the cylinder. In a preferred embodiment the cylinder or the piston is fastened on the rigid plate at the opposite side (preferably to bottom side) of the primary assembly and this secondary assembly is connected to a stationary support construction via an elastic material capable of moderating movement of this secondary assembly. In this construction mechanical force in the form of vibration is transferred to the primary assembly (and to the stained fabric and movable body within the test container) by the weight displacement and/or acceleration by which ever of the cylinder or piston, which is not connected to the rigid plate. This displacement is achieved by moving the piston or cylinder relatively to the other elements of the secondary assembly by means of a hydraulic or pneumatic system. Such devices for providing mechanical force are commercially available e.g. from Copenhagen Vibrator Product, Denmark.

An alternative to the piston-cylinder construction is a device comprising an electro engine spinning a mass element, such as a driving a wheel, having a heterogeneous mass distribution, said device connected to the primary device and to a stationary support construction via a rigid plate. To moderate movement the rigid plate may be connected to a stationary support via an elastic material, such as rubber. When the mass element spins heterogeneous mass distribution will cause repetitive displacement of mass and will confer vibration to the primary assembly and the container holding the test sample.

In another embodiment the assembly comprises a magnetic device which comprises a permanent magnet or an electromagnet. In the case of a permanent magnet such as a Neodymium Iron Boran magnet, the magnetic device also comprise means for providing movement of the magnet relative to a magnetizable body comprised in the container of the primary assembly

applying an alternating magnetic force to a magnetizable body present in the container.

In a specific embodiment this secondary assembly preferably is a carousel construction comprising

(a) a horizontal rotatable support surface comprising means for fastening, in a position different from the rotational center, a container containing a movable and magnetizable body and a least one opening covered with the stained coherent fabric,

(b) a permanent magnet connected to the axis, enabling variation in the magnetic field applied to the container upon rotating the support and

(c) means for rotating either the magnet, the support surface or both so as to create a relative movement between magnet and support surface.

The magnet is preferably placed in a fixed position so that the support surface is the only movable part and so that the test container(s) upon rotation is allowed to come into sufficient proximity of the magnet so that a magnetic field is applied to the body in the test container, which is sufficient to move the body within the container towards the magnet.

The invention also relates to the use of a coherent stained fabric as cover and/or lid on an array of at least two, preferably at least 24, more preferably at least 96 containers having a volume of less than 10 ml for testing cleaning ingredients.

Special method embodiments

Although for most enzymes acting directly on the cellulose in textile, severe mechanical stress of the stained surface is important, we have also found that when testing cleaning effect of non cellulolytic enzymes such as protease, lipase amylase and oxidoreductase on stained fabric, the mechanical stress provided by repeatedly moving the sample liquid against the fabric surprisingly gives test results which is very similar to results obtained in real laundry processes. This may be achieved by shaking or vibrating containers holding the liquid samples and moving the liquid against the stained fabric. Combined with the finding that a coherent stained fabric may be used to test liquid samples in more than one container simultaneously by using the fabric as a lid and seal on the containers (see description of the primary assemblies, *supra*) these findings provide a powerful tool for automated testing of these enzymes. Accordingly, the present invention also relates to a method for testing cleaning effect of a non-cellulolytic enzymes said method comprising:

(a) Preparing liquid samples comprising the non-cellulolytic enzyme in an assembly according to any of the claims 30-40, with the proviso that the container does not contain a solid body capable of moving inside the container,

(b) repeatedly applying liquid sample to the stained fabric,

(c) evaluating the cleaning effect of applying solution on the stained fabric.

This embodiment of course also encompasses within step (a) use of all primary assemblies as described, supra for testing cleaning ingredients.

EXAMPLES

Example 1

Effect of mechanical stress on stain removal performance:

Light reflectance values of fabrics having different stains washed in a Launder-O-Meter at low and at high mechanical conditions.

Preparation

Stained fabrics were obtained from Equest Market Research Limited, Equest House, Greencroft Industrial Park, Annfield Plain, Stanley, Co. Durham, DH9 7YB, England, a Launder-O-Meter with 500 ml beakers was obtained from Roaches England LTD, Washtec and a liquid detergent Ariel Futur was used.

Test procedure

A wash solution of 200 ml city water with 1.3 ml Ariel Futur liquid detergent and 5 stained fabrics (4 x 4 cm) was added to each beaker. In beakers wherein low mechanical stress were to be tested no bodies were added, while in beakers wherein high mechanical stress were to be tested were added 10 Teflon coated star magnets (24 mm diameter) and 30 steel nuts (25 mm diameter). The stained fabric were washed in the Launder-O-meter for 30 minutes wash at 40° C with or without.

Evaluation of cleaning effect

Light reflectance values of the stained fabrics were measures by a Spectrafash 500 equipment from "datacolor international".

L-values	burnt beef	make-up	grass/mud	chocolate ice-cream
original stain	42.9	59.2	54.1	64.1
washed with low	57.4	63.5	72.8	74.1

mechanical stress				
washed with high mechanical stress	76.1	81.0	84.2	88.1
clean fabric	92.8	92.8	92.8	92.8

noise level about +/- 1.5 units

From this experiment it can be observed that mechanical stress to the stained fabric has a significant effect on the cleaning effect of the detergent ingredients.

Example 2

A test device was developed according to figure 1, wherein 1 is a movable permanent Neodymium Iron Boran magnet (20 x 2.5 x 1.3 cm) with 12200 Gauss strength; 2 = a stained fabric; 3 are wells of a 96 well micro plate (350 ul volume size); 4 = liquid sample; 5 are magnetizable rectangular stainless steel bodies (3 x 1.6 x 1.9 mm) which by movement of the magnet and application of magnetic force jumps up, rolls and scrapes against the stained fabric while simultaneously depositing liquid sample on the fabric.

Example 3

The test device of example 2 was used to test a carbohydrate hydrolyzing enzymes. A knitted cotton fabric coated with tamarind seed flour and carbon black particles was used as stained fabric, which was cut to a dimension fitting a 96 well micro plate.

Preparation

150 ul wash liquor containing 1 mg Ariel Futur liquid detergent and 4 rectangular stainless steel bodies (3 x 1.6 x 1.9 mm) was added to each well. Every second array wells also

contained 1 ppm of a Xyloglucanase from *Bacillus licheniformis*.

Process

The micro plate was heated to 40°C in a water bath and after assembling the stained fabric to the top of the filled micro plate, a strong magnet was moved every two seconds over the stained fabric for 30 minutes.

Result

Differences in cleaning effect between wells containing the enzyme and wells in which the enzyme is absent was visually detectable as shown in figure 2, wherein "0" denotes no enzyme and "1" denotes presence of 1 ppm of enzyme in the test solution. This experiment also shows the benefits of using one coherent stained fabric covering all 96 wells of the micro plate, because although all 96 results are to be measured from this stained fabric, the fabric has a size which is much more easily handled in the measuring procedure, than if 96 individual pieces of stained fabric were to be measured.

Example 4

In this example the correlation between real wash performance and using the method of the invention was evaluated for two different enzymes.

Enzyme cleaning effect using the method of the invention was evaluated as described in example 3 using 1 ppm enzymes levels and 8 replica samples per enzyme. The two different enzymes were A: Xyloglucanase from *Bacillus licheniformis and* B: Xyloglucanase from *Bacillus firmus*. The cleaning effect was evaluated by measuring the light reflectance (L-values) of the portions of stained fabric subjected to the test solutions and the mechanical stress. A Spectrafash 500 equipment from "Datacolor International" was used.

Enzyme cleaning effect using a real washing process was evaluated by washing Tamarind seed flour/carbon black stained fabric (5 x 5 cm swatches) in a Miele W715 washing machine, at 40° C, short cycle together with 0.5 kg realistic items. The same levels and types of liquid detergent and enzyme were used and for each enzyme 8 replica washes were preformed. The cleaning effect was evaluated by measuring L-values of the stained fabric after wash.

Results

The results as given in the table below shows and excellent correlation between using the method and device of the invention and realistic washing processes

	L-values of treated stains			
Enzyme	micro plate system (SD)		washing machine (SD)	
A	62.2	0.7	65.2	1.6
B	57.0	0.5	58.9	0.7
no enzyme	54.3	0.9	50.0	1.5

Example 5:

A primary assembly suitable for use in testing cleaning ingredients was prepared as shown in figure 3(A), wherein (1) are wells of a micro plate having connecting parts between wells which are lowered as compared to the edges of the wells; (2) is a lid having an essentially plane surface facing the micro plate well; (3) the contact zone between the lid and the well, wherein the fabric, when inserted between (1) and (2), is compressed to form a gasket seal. This assembly provides an excellent seal and greatly prevents or inhibits the liquid sample from one well to diffuse or migrate to a neighboring well. This assembly also provides that the stained fabric surface accessible to liquid

sample and mechanical stress is at least the same as the area of the opening of the well.

Example 6

Another primary assembly suitable for use in testing cleaning ingredients was prepared as shown in figure 3(B), wherein (1) are wells of a micro plate having connecting parts between wells which are lowered as compared to the edges of the wells; (2) is a lid having elevations fitting into depressions formed around the wells in the micro plate; (3) is the contact zone between the lid and the micro plate well, wherein the fabric, when inserted between (1) and (2), is compressed to form a gasket seal. This assembly provides a slightly poorer sealing effect than the assembly of example 5, but has the advantage that the micro plate part of the contact zone is subjected to less wear upon use and has increased durability. Also the this assembly provides for the stained fabric surface accessible to liquid sample and mechanical stress being at least the same as the inner area of the well opening.

Example 7

Another primary assembly suitable for use in testing cleaning ingredients was prepared as shown in figure 3(C), wherein (1) are wells of a micro plate having connecting parts between wells which are lowered as compared to the edges of the wells; (2) is a lid having elevations fitting into the opening of the well (like a cork) and to depressions formed around the well in the micro plate; (3) is the contact zone between the lid and the micro plate well, wherein the fabric, when inserted between (1) and (2), is compressed to form a gasket seal. This assembly provides a slightly poorer seal than the assembly of example 5, but as in example 6 possesses increased durability because the micro plate part of the contact zone is subjected to less wear upon use.

Example 8

Another primary assembly suitable for use in testing cleaning ingredients was prepared as shown in figure 3(D), wherein (1) are wells of a micro plate having connecting parts between wells which are heightened as compared to the edges of the wells; (2) is a lid having elevations fitting into the opening of the wells (like a cork); (3) is the contact zone between the lid and the micro plate well, wherein the fabric, when inserted between (1) and (2), is compressed to form a gasket seal.

Example 9

Another primary assembly suitable for use in testing cleaning ingredients was prepared as shown in figure 3E, which is similar to the assembly of example 6, with exception that the elevations of the lid forms curves instead of straight tilted lines. This provides an increased durability.

Example 10

An another primary assembly suitable for use in testing cleaning ingredients was prepared as shown in figure 3F, which is similar to the assembly of example 8, with exception that the elevations of the lid forms curves instead of straight tilted lines. This provides an increased durability.

Example 11

An another primary assembly suitable for use in testing cleaning ingredients was prepared as shown in figure 4, which is similar to the assembly of example 6, with the exception that this assembly was made in the standard format of a 96 well plate with standard size of wells and distances between wells. Accordingly, this assembly will fit into standard equipment developed for this format. (1) is the support lid, (2) is the stained fabric and (3) is the micro plate.

Example 12

A secondary assembly which further comprises a device for providing mechanical force to the stained fabric was prepared as shown in figure 5. In this secondary assembly a primary assembly of the type described in example 11 was fastened to a NTK 15 or NTK 25 type pneumatic flask vibrator available from Copenhagen Vibrator Products, Industrivej 15, 4652 Hårlev, Denmark. This construction is showed in figure 5, wherein the primary assembly (1) is mounted with bolts (9) on the top side of a rigid plate (2) and a cylinder (3) is mounted on the down side of the rigid plate (2), The cylinder (3) surround a piston (4) with mass elements (5), capable of moving in the longitudinal direction of the cylinder (3) by applying compressed air to valves or apertures (6) in the cylinder. This rigid plate (2) is rested on a stationary support (7) via rubber connectors (8) which is capable of moderating the vibrating force.

Example 13

Five different microbially derived xyloglucanases; A, B, C, D, E and one cellulase F were tested with (+) and without (-) using steel beads to provide mechanical stress using the test plate of example 11 fitted in the assembly of example 12. Each enzyme were diluted to give a final concentration of 1/16,1/8, ¼, ½, 1, and 2 ppm in the 96 well plate containing 6.7 g/l Ariel Color liquid detergent in 15°dH H_2O in a final volume of 160 µl. Eight repeats of each dilutions were carried out. A fabric stained with tamarind gum and carbon black was used. Each well in which high mechanical stress were to be tested contained 3x2 mm steel beads, wells wherein low mechanical stress where to be tested contained no steel beads. Using this construct the stained fabric was which was washed for 30 min at 26°C under vibration by the vibrating device.

The cleaning results were evaluated by measuring light reflectance of the cleaned fabric.

Results

Enzyme dose	Enzyme											
µg/ml	A-	A+	B-	B+	C-	C+	D-	D+	E-	E+	F-	F+
0	1718	1882	1694	1880	1884	1919	1654	2012	1552	2105	1648	1929
0.0313	1671	2003	1679	2025	1670	2015	1925	1830	1836	2116	1793	1940
0.0625	1745	1992	1859	2215	1620	2046	1650	1970	1746	1977	1706	1924
0.125	1808	2168	1914	2105	1605	1903	1746	2056	1686	1989	1698	1946
0.25	1741	2343	1693	2321	1814	1978	1805	2106	1775	2256	1687	1997
0.5	1862	2524	1867	2381	1799	2224	1794	2186	1607	2312	1714	1848
1	1884	2639	1804	2468	1784	2086	1808	2127	1830	2156	1747	1836
2	1815	2424	1743	2320	1768	2190	1712	2234	1693	2298	1685	1833

The results shows that for each enzyme treatment with the steel beads increases removal of stain, similar to results obtainable for real washing processes. Also a ranking of the different enzyme efficiency could be was deducted from the results, rendering enzyme A the most efficient enzyme.

Example 14

In order to show the applicability of the system to other enzymes a series of proteases were tested using the assembly of example 12 using both commercial 96 well test plate and the custom made test plate of example 11. The proteases were mixed in amounts of 0, 5, 10, 20 and 40 nanomoles per liter to samples of a commercial Omo color detergent dissolved in 15°dH water yielding a pH of about 10.4 in the solution. The test samples were transferred to the wells of the test plates, and all well openings in each test plate were subsequently covered by one single coherent EMPA 117 stained test fabric and a lid. The wells of the test plate did not contain a body for providing mechanical force.

The test plates were then mounted on the assembly of example 12 and vibrated at 30°C, and after this treatment each part of the test fabric corresponding to single samples was evaluated. For both plates a clear indication of samples having a high protease concentration was observed, so that samples having higher protease concentrations had removed more stain from the fabric in an area corresponding to the circumferences of the wells. However, for the commercial plate it was evident that the test samples had spread beyond the area of the stained fabric which was circumferenced by the edge of the well (i.e. the area of the opening of the well) so that stain removal was also seen outside the areas of the stained fabric directly exposed to the test samples. Accordingly the test samples were leaking from the wells or migrated through capillary effects to areas of the stained test fabric not directly covering the opening of the wells. This phenomenon obscured the results of the test. The results of using a stained fabric with a commercial 96 well test plate can be seen in figure 6. In figure 6 A-D represents different proteases tested at different concentrations.

Using the custom made test plate, the test samples were effectively confined to act only on the area of the stained fabric covering the opening of the well. Consequently very clear cleaning results were obtained, where the effect of different concentrations could easily be identified. Moreover it was possible to visually determine that protease D had better cleaning performance than protease A. This result also show that for proteases and other non-cellulose hydrolyzing enzymes application of mechanical force by means of a solid body colliding with the stained fabric is not necessary for these enzymes.

PATENT CLAIMS

1. A method for testing cleaning effect of a compound or compositions thereof said method comprising:

(a) Preparing a liquid sample of less than 10 ml comprising the test compound,

(b) applying liquid sample to a stained surface,

(c) applying mechanical stress to the stained surface by contacting it with a body present in the liquid sample,

(d) evaluating the cleaning effect of applying solution and mechanical stress on the stained surface.

2. The method of claim 1, wherein the test compound is selected from enzymes, enzyme stabilizers, enzyme inhibitors, enzyme enhancers, enzyme co-factors, builders, builder systems, bleach systems, bleach activators, metal-containing bleach catalyst, optical brighteners, nonionic -, anionic -, cationic -, zwitterionic and amphoteric surfactants, fabric softening agents, softening clays, clay flocculants, dye-transfer inhibiting agents, polymeric soil release agents, clay soil removal agents, anti-soil redeposition agents, polymeric dispersing systems, chelating agents, alkoxylated polycarboxylates, perfumes, perfume systems, carrier systems, dyes and pigments, fabric care agents and color care agents.

3. The method of claim 2, wherein the enzymes are alkaline.

4. The method of claim 1, wherein the liquid sample has a volume selected from 5 - 95% of the volume of 3.7 ml, 320 µl, 160 µl, and 14 µl, respectively.

5. The method of claim 1, wherein the surface is inorganic selected from metal, ceramic, glass, enamel concrete, rock,

marble, gypsum or composite combinations thereof or organic selected from plastic, rubber, wood, paper, leather, fur, paint or fabric.

6. The method of claim 5, wherein the surface is a fabric.

7. The method of claim 6, wherein the fabric is made from natural plant fibers, animal based fibres or synthetic fibres or combinations thereof.

8. The method of claim 7, wherein the fabric is woven, non-woven, soft or stiff.

9. The method of claim 8, wherein the fabric is a cellulose containing fabric selected from textiles (woven), and tissues (non woven) or an animal based fabrics, preferably wool.

10. The method of claim 1, wherein the stain comprises a traceable compound or composition associated to the surface.

11. The method of claim 10, wherein the stain is a traceable compound or composition associated to the surface.

12. The method of claim 10, wherein the traceable compound is selected from light absorbing dyes, fluorescent dyes, radioactive compounds, reactive compounds and catalysts or activators capable of performing measurable interaction with substrates.

13. The method of claim 10, wherein the traceable compound is comprised in a particulate composition, preferably carbon particles or iron oxide particles.

14. The method of claim 10, wherein the traceable compound is comprised in a soiling composition.

15. The method of claim 14, wherein the soiling composition is selected from naturally occurring soiling and processed soilings thereof.

16. The method of claim 15, wherein the soiling composition is a natural occurring soiling selected from grass, mud, clay, coffee, tea, blood, egg, lard and moulds

17. The method of claim 15, wherein the soiling composition is a processed naturally occurring soiling selected from butter, processed meat, dyed lard, oil, make up, spice blends, processed tomatoes (ketchup or puree), chocolate, ice cream, cacao, baby food, refined protein compositions, refined polysaccharide compositions, refined fatty acid compositions, refined triglyceride compositions.

18. The method of claim 1, wherein the body comprises a metal, more preferably a ferromagnetic metal, more preferably iron or alloys thereof.

19. The method of claim 18, wherein the body has a surface comprising at least one edge or corner.

20. The method of claim 1, wherein the stained surface is placed at the top or the bottom of the container.

21. The method of claim 20, wherein the stained surface functions as a cover on an opening in the container.

22. The method of claim 1, wherein the mechanical stress is applied by moving the body against the surface.

23. The method of claim 22, wherein the liquid sample applied to the stained surface by moving the body against the surface and depositing liquid sample adhering to the moving body.

24. The method of claim 23, wherein the body is moved by repeatedly applying a force to the body.

25. The method of claim 24, wherein the force is an oscillating force selected from randomly oscillating force and periodically oscillating force.

26. The method of claim 24 and 25, wherein the force is selected magnetic force, electromagnetic force, electrical force, mechanical force or a combination thereof.

27. The method of claim 26, wherein the force is a magnetic force applied to a magnetizable body by moving a magnet relatively to the container containing the body.

28. The method of claim 26, wherein the force is a mechanical vibration force applied to an assembly comprising body, container and stained fabric.

29. A device suitable for testing cleaning effect of a composition, said device comprising:

(a) at least one container having a volume of less than 10 ml, preferably less than 2 ml, most preferably less than 0.2 ml ml,

(b) at least one body capable of moving inside the container,

(c) at least one stained surface, preferably a stained fabric and

(d) means for providing movement of the body relatively to the stained surface.

30. An assembly suitable for use in the device of claim 29 comprising at least one container and a stained coherent fabric, wherein the container comprises at least one opening covered with the stained coherent fabric.

31. The assembly of claim 30, comprising an array of containers, wherein each container comprises one or more openings and wherein the stained coherent fabric covers at least one of the openings in each container.

32. The assembly of claim 31, further comprises least one body in each container.

33. The assembly of claim 31, wherein the array of containers is a micro plate selected from 24, 96, 384 or 1536 well micro plates.

34. The assembly of claim 30 , wherein the stained coherent fabric have a dimension within 0.2-10 cm by 0.2-15 cm, preferably a dimension enabling the stained fabric to cover all the wells in the micro plate.

35. The assembly of claim 30, further comprising a support lid located in the assembly so that the coherent stained fabric is positioned between the support lid and the container opening.

36. The assembly of claim 31, wherein the interconnecting surface between containers in an array and the surface of the support lid facing the fabric and the container opening are unparallel.

37. The assembly of claim 30, further comprising means for providing mechanical stress to the stained fabric.

38. The assembly of claim 37, wherein the means for providing mechanical stress comprises a piston-cylinder constructions, which by movement of the piston cylinder constructions confers vibrational force to the container.

39. The assembly of claim 37, wherein the means for providing mechanical stress comprises an electro engine connected to the container, the engine spinning a mass element, wherein the mass is heterogeneously distributed around the spinning axis whereby the spinning of the mass cause a repetitive displacement of mass conferring vibrational force to the container.

40. The assembly of claim 37, wherein the means for providing mechanical stress comprises a permanent magnet and means for providing movement of the magnet relative to a magnetizable body comprised in the container.

41. Use of a coherent stained fabric as cover on an array of at least two, preferably at least 24, more preferably at least 96 containers for testing cleaning ingredients.

42. Use of the assembly of any of the claims 30-40 for testing cleaning ingredients.

43. A method for testing cleaning effect of a non-cellulolytic enzymes comprising:

(a) Preparing liquid samples comprising the non-cellulolytic enzyme in an assembly according to any of the claims 30-40, with the proviso that the container does not contain a solid body capable of moving inside the container,

(b) repeatedly applying liquid sample to the stained fabric,

(c) evaluating the cleaning effect of applying solution on the stained fabric.

44. The assembly of claim 40, wherein the assembly comprises:

(a) a rotate able horizontal support surface mounted on a vertical axis, said surface comprising means for fastening the container at a position different from the rotational centre and

(b) a permanent magnet connected to the axis, enabling variation in the magnetic field applied to the container upon rotating the support.

ABSTRACT

Title: Automated mechanical stress assay for screening cleaning ingredients

A method for testing cleaning effect of a compound or compositions is disclosed. The method comprises:

(a) Preparing a liquid sample of less than 10 ml comprising the test compound,

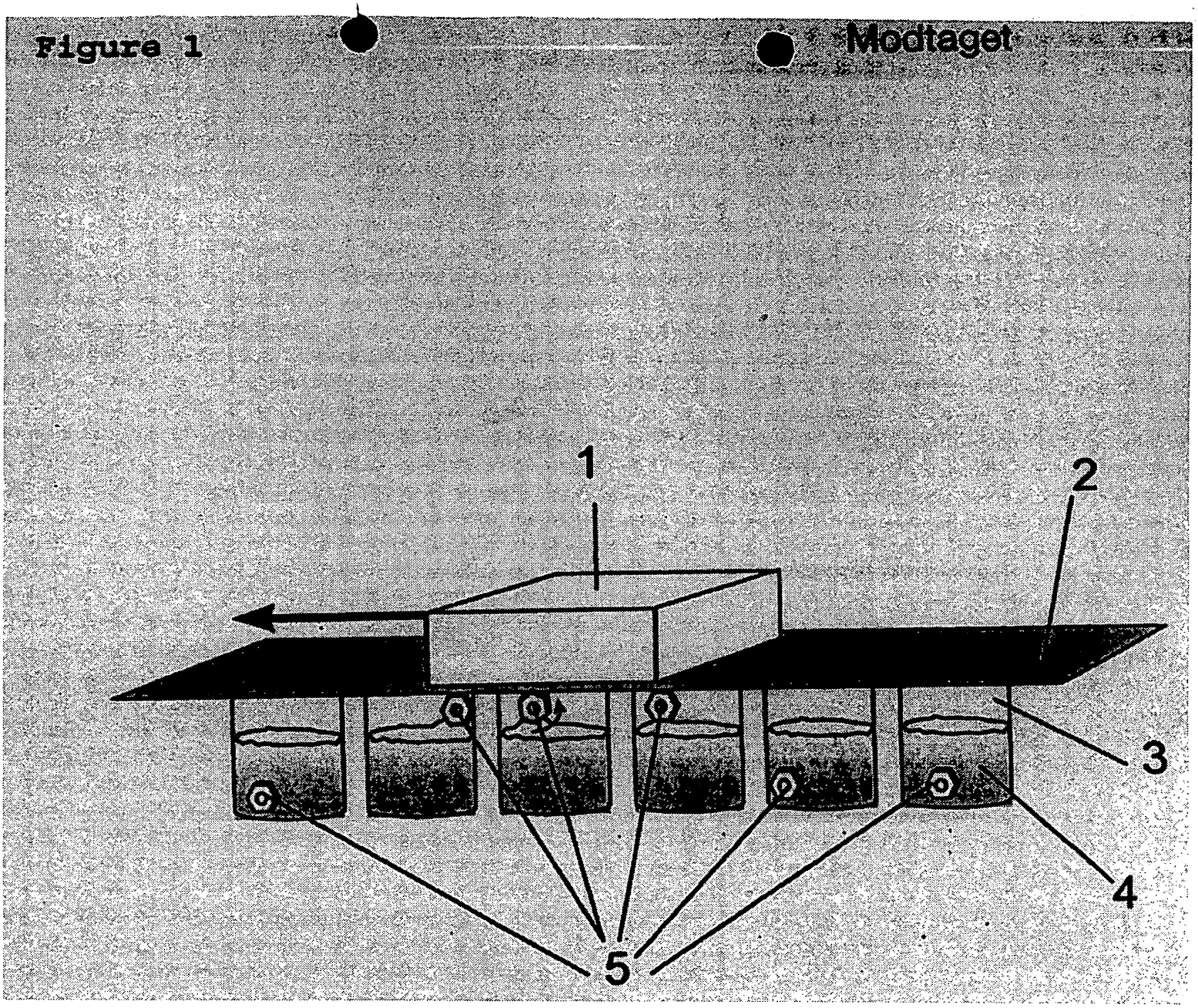
(b) applying liquid sample to a stained surface,

(c) applying mechanical stress to the stained surface by contacting it with a body present in the liquid sample,

(d) evaluating the cleaning effect of applying solution and mechanical stress on the stained surface.

Modtaget

Figure 1

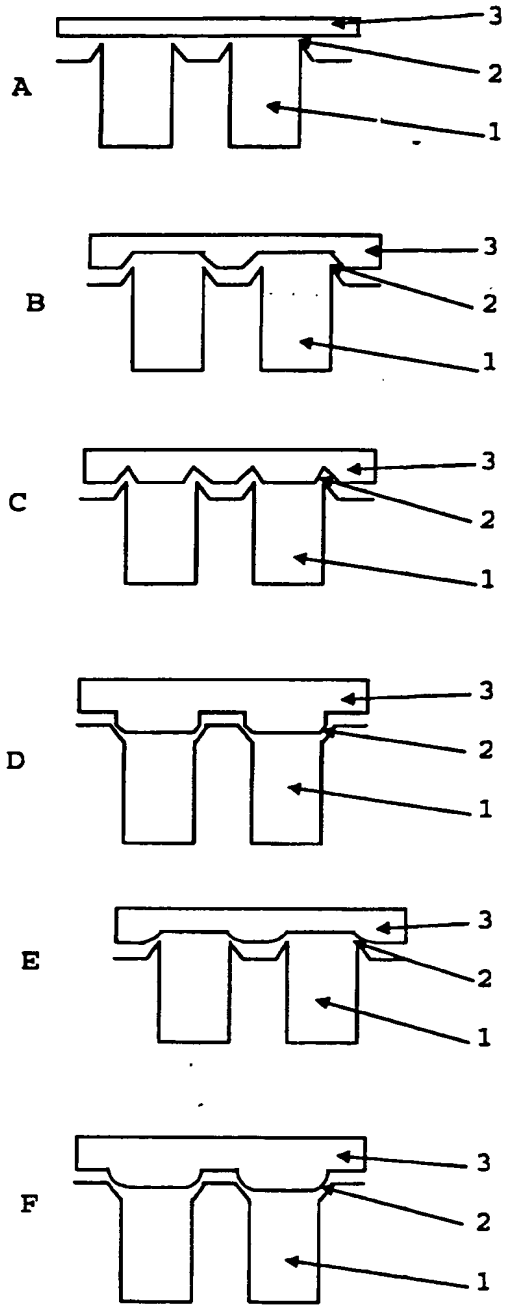


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Figure 3



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Figure 4

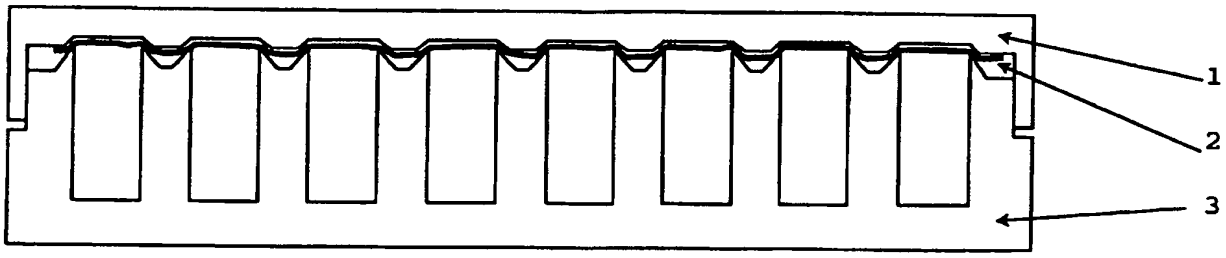
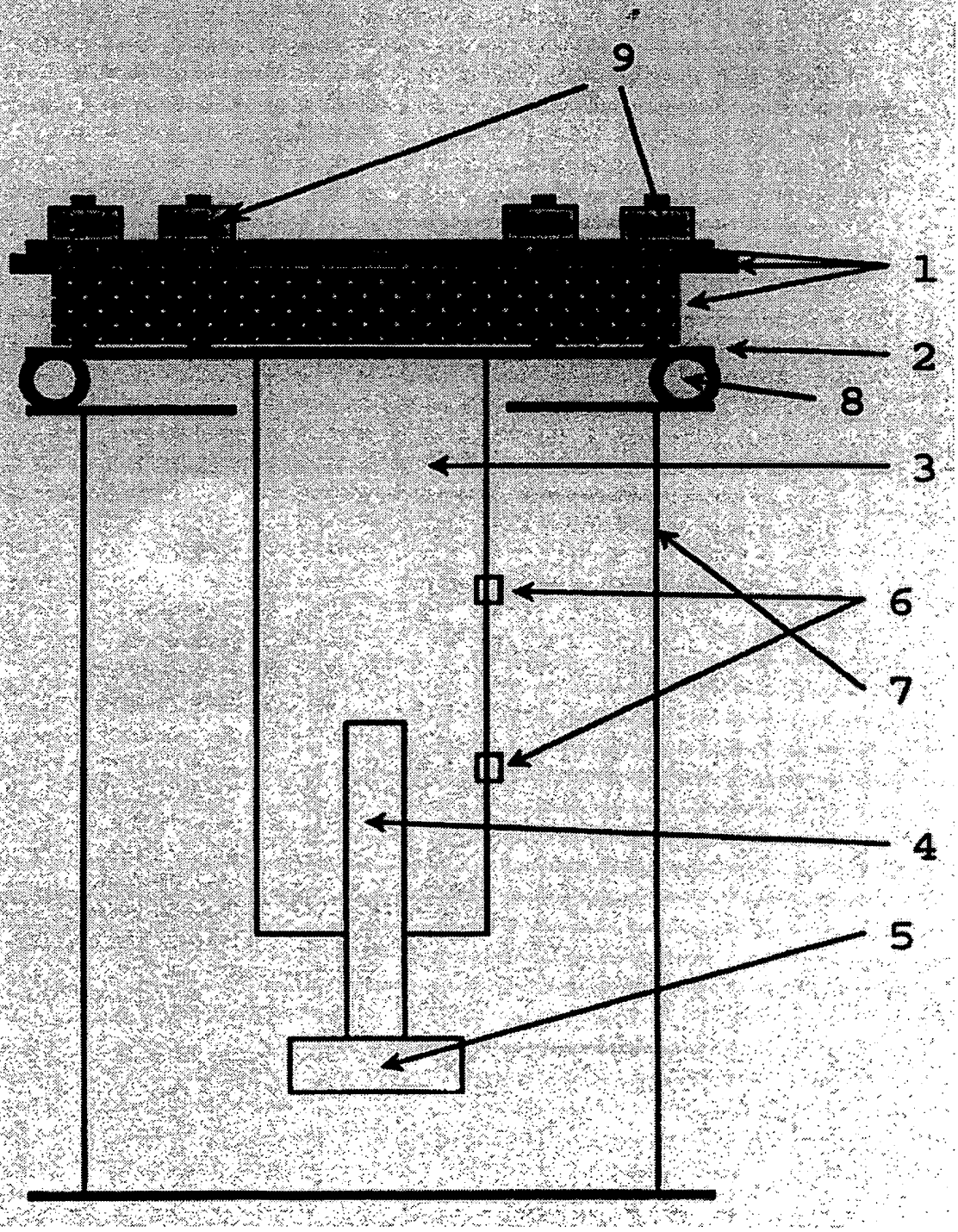
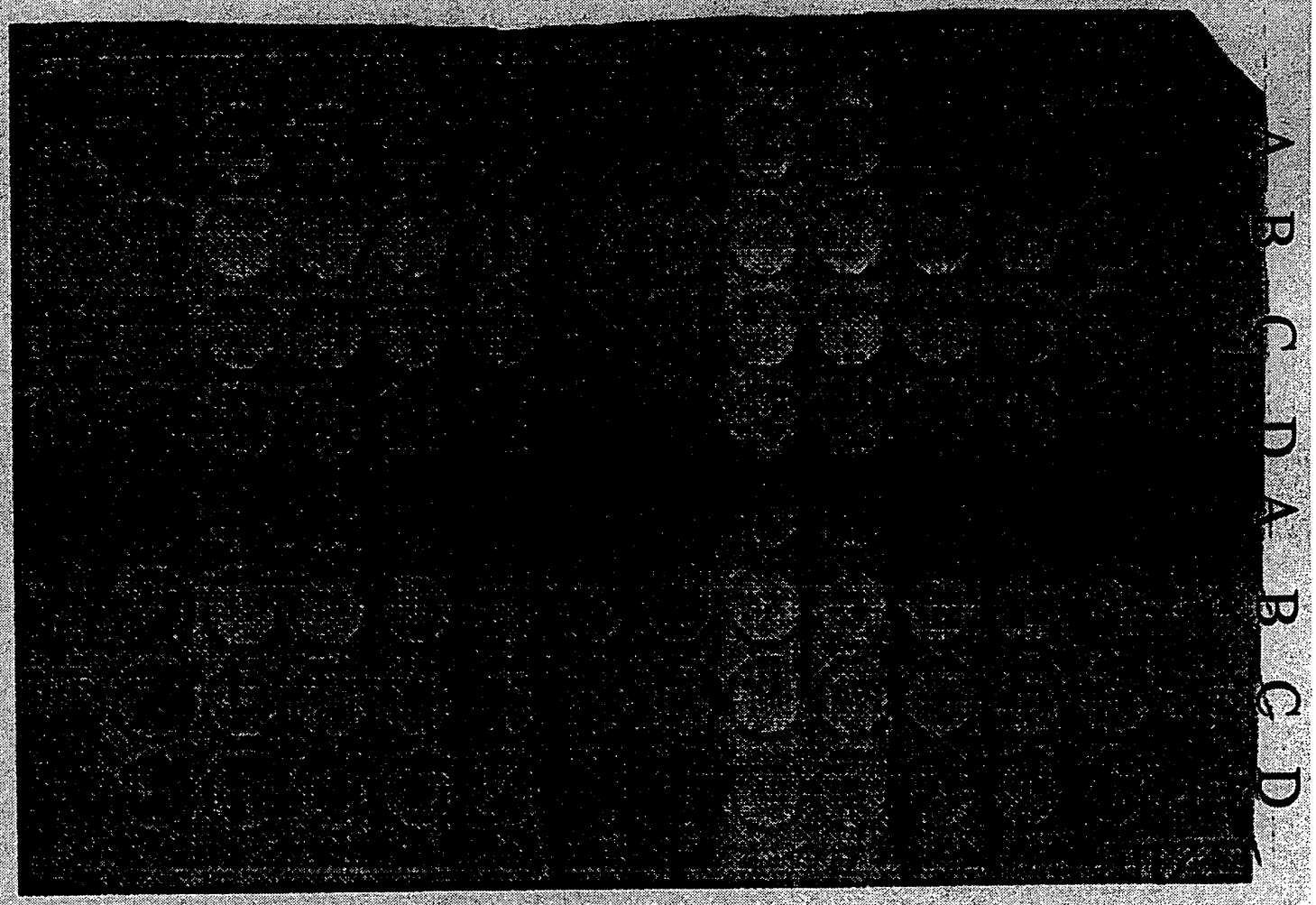


Figure 5

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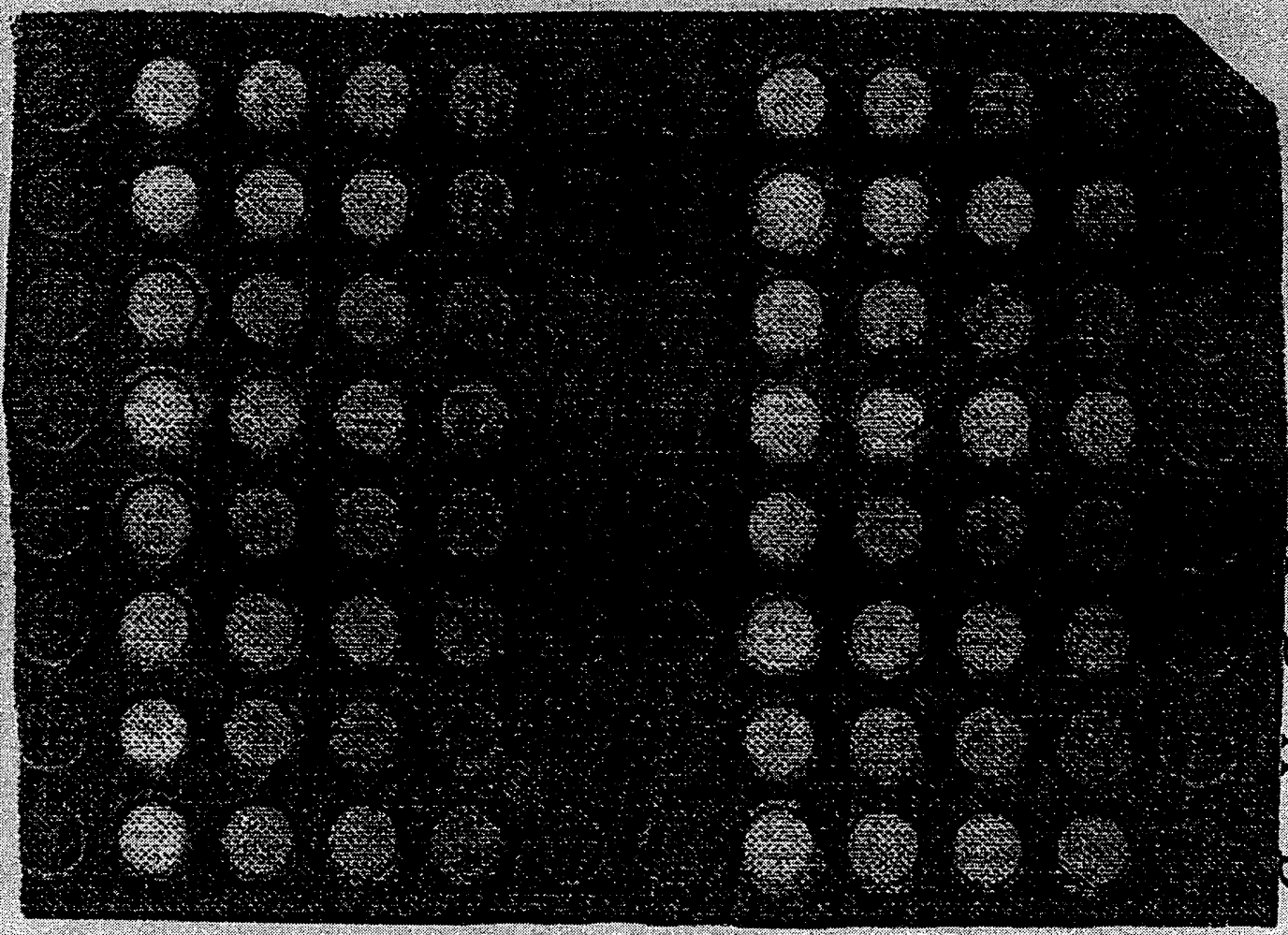


Modtaget



0 40 20 10 5 0 0 40 20 10 5 0

Nanomoles per liter


A D A D A D A D
0 40 20 10 5 0 0 40 20 10 5 0
Nanomoles per liter